CENTROSOME AND SPHERE IN THE EGG OF UNIO.

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In this paper I shall present some observations on the relation of the centrosome to the sphere, and of both to the cytoplasm. It is still undecided, I think, what is to be called centrosome and what sphere in all cases (*e.g.*, see MacFarland,[1] McMurrich,[2] and Haecker[3]); and it is still possible to take either side in the controversy as to whether the centrosome is a unique and permanent organ of the cell or not. If the centrosome is not a permanent organ, it must, at some phase in the life cycle of the organism, lose its identity as centrosome and be replaced. Hence, sufficiently exhaustive observation must reveal lack of continuity. If it can also be shown that centrosomes may arise at various points in the cell, the centrosome loses its place as a unique and permanent organ of the cell. If, finally, any of the products of division of the centrosome become other *formed* elements of the cell, the same conclusion follows. It is this last line of argument on which I shall lay most weight in this paper.

In the *metaphase of the first maturation spindle* both asters possess the following structure (Figs. 1 and 2):

a. In the exact center is a minute black speck, the centrosome, either round or dumb-bell shaped, into which are "inserted" the central ends of some, at least, of the rays.[4]

b. There are two concentric spheres, corresponding to the "medullary" and "cortical" zones of Van Beneden, bounded by microsomes regularly arranged on the rays. The ground

[1] MacFarland, Dr. F. M., "Celluläre Studien an Mollusken-Eiern," *Zool. Jahrbücher, Abth. für. Anat. und Ontogenie der Thiere.* Bd. x, Heft 2. 1897.

[2] McMurrich, J. Playfair, "The Yolk-lobe and the Centrosome of Fulgur Carica," *Anat. Anz.* Bd. xii, Nr. 23. 1896.

[3] Haecker, V., "Ueber den heutigen Stand der Centrosomafrage," *Verhandlungen der Deutschen Zool. Ges. zu München.* 1894.

[4] The sections, 5 μ thick, were stained in Heidenhain's iron haematoxylin and Bordeaux red.

substance of the inner sphere is quite dense, and takes the red stain (Bordeaux red) strongly.

c. The radiating fibers are simply an arrangement of the cytoplasmic network, with all that this implies.

d. Microsomes are found on all fibers of the asters and of the spindle, with the possible exception of the central spindle. They are certainly least conspicuous on the central spindle.

In the *late anaphase of the first maturation spindle* the centrosome has divided into two, and the inner sphere is bounded by a *continuous membrane*, into which the central ends of the

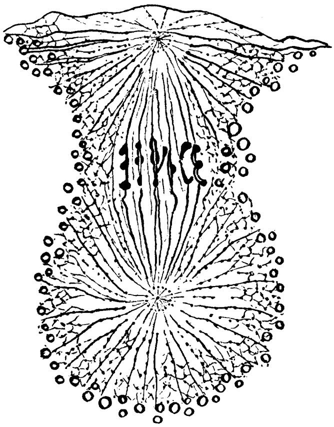

FIG. 1. — First Maturation Spindle of Unio.

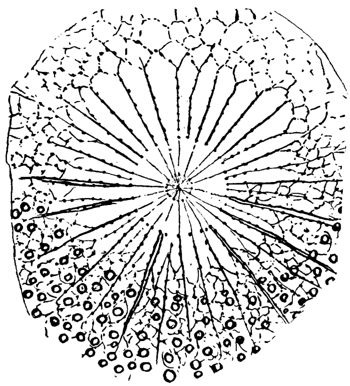

FIG. 2. — Horizontal Section through the Outer Aster of the First Maturation Spindle of Unio in the Stage of Fig. 1.

rays are inserted. This membrane (*cf.* Fig. 6) is produced in part by the fusion of the inner stratum of microsomes, but chiefly by the peripheral accumulation of the ground substance of the inner sphere. The substance of the mantle fibers is heaped up in the cortical zone, and the fibers of the central spindle exhibit large, deeply staining microsomes.

In the *early telophase of the first maturation spindle* the centrosomes are extremely large, and each is composed of a group (at least four) of densely black granules. *The centrosomes are united to the membrane of the inner sphere by a few irregular threads which are not part of the system of radiations* (*cf.* Fig. 6). The rays of the aster and the fibers of the central spindle are studded with enormous closely set black microsomes, the intervening substance staining faintly in Bordeaux.

In the *concluding phases of the telophase*, the inner chromosomes and sphere move towards the surface, drawing out into rays the protoplasm of the degenerating aster. In this stage a new set of fibers radiates in a fan-shaped manner from the sphere to the surface. These radiations then disappear entirely and in place of the two centrosomes but one is found within the inner sphere. In some cases the inner sphere may disappear, and then the centrosome cannot be distinguished from

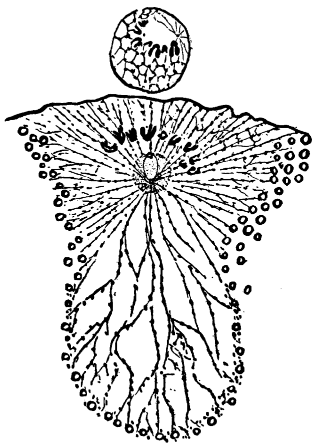

FIG. 3. — Prophase of the Second Maturation Spindle in Unio. Origin of the Central Spindle.

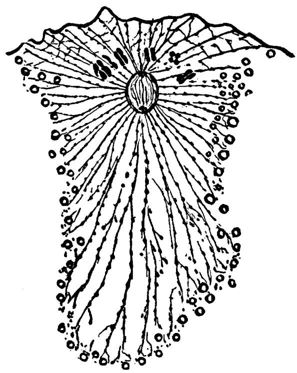

FIG. 4. — Prophase of the Second Maturation Spindle in Unio. Second Stage in Origin of the Central Spindle.

cytomicrosomes. This concludes the telophase of the first maturation division. Without any pause begin

The Prophases of the Second Maturation Spindle. The formation of the second maturation spindle may take place in either of two ways, in both of which, however, the centrosome phenomena are essentially the same. In the first method (Figs. 3–5) the central spindle is formed at the point at which the centrosome remains after the formation of the first polar globule. The central spindle in this method is generally radial in position, though it may lie at any angle. The second method is preceded by the disappearance of the inner sphere, and the horizontally placed central spindle together with the chromosomes sink in towards the center of the egg. The spindle later rotates into position.

In the description of the centrosomes I shall follow the first method entirely. See Figs. 3–5, radial sections through the entire central spindle, and 7*A–E*, horizontal sections through the outer centrosome and spheres drawn on a much larger scale.

The prophase of the second maturation spindle is inaugurated by the origin of a new set of radiations around the sphere (Fig. 3). Within the sphere are two centrosomes united by delicate threads, the beginnings of the central spindle. Each centrosome is composed of several granules. The radiations are generally attached to the sphere, but they can sometimes be traced in part to the centrosomes.

As the spindle elongates, the sphere becomes elliptical, as though stretched by the central spindle, the ends of which abut against it (Fig. 4). The centrosomes have become still more subdivided, and have increased in bulk. The fibers of the central spindle have also increased greatly in number and distinctness. The rays surrounding the sphere are beginning to disappear. They are, plainly, inserted in the elliptical sphere. This is the stage which MacFarland has figured in such detail for Diaulula. He calls the whole sphere the centrosome, because the rays are inserted in it. The history of this body, which was not sufficiently investigated by MacFarland, makes it plain that it is not the *centrosome*, but the *inner sphere*. The insertion of rays is therefore in itself no criterion of a centrosome.

In a slightly later stage the rays have been entirely resolved into vesicular cytoplasm ; and the sphere has been stretched out into the peripheral fibers of the central spindle. The centrosomes are yet more subdivided (Fig. 7*A*), and the mantle fibers are just beginning to form.

Now follow some very important centrosome phenomena ; when the spindle has elongated a little more the radiations of the asters begin to develop, those of the inner aster before those of the outer in the radial position of the spindle. In a radial section through such a stage, one is inclined at first to think that the inner centrosome has disappeared. More careful observation shows, however (Fig. 5), that the center of

the inner aster is occupied by an extraordinarily minute centrosome lying in a sphere. In the metaphase the outer aster has exactly the same structure (Fig. 7*E*). Later yet the inner sphere of both asters is bounded by a membrane (Figs. 7*F* and 6) formed in precisely the same way as the homologous structure of the first maturation spindle. In this stage the centrosome is much larger than at the metaphase. *What, now, has become of the compound centrosomes of earlier stages, and what is the origin of the concentric spheres?*

Figures 7*A* to *F* illustrate the changes undergone ; 7*A* to *E* are drawn from horizontal sections of the outer aster, and 7*F*

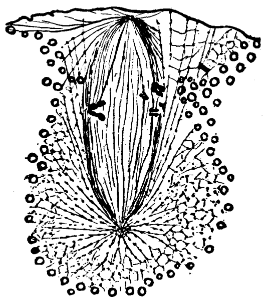

FIG. 5. — Later Stage of the Second Maturation Spindle in Unio.

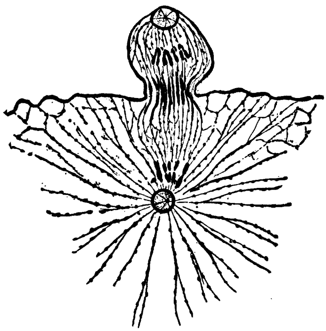

FIG. 6. — Telophase of the Second Maturation Division in Unio.

and 6 from radial sections of the inner aster in the metaphase and telophase of the spindle, respectively. The condition seen in Fig. 7*A* has been reached by growth and division of a single small centrosome ; this figure represents a horizontal section through the outer centrosome of the stage of Fig. 5. The next four figures carry us to the beginning of the metaphase. They show two processes taking place : (1) The subdivision of the relatively large centrosome granules and their distribution in the form of a sphere ; and (2) the increase of the red-staining substance in which the granules are imbedded. The peripherally distributed granules become the stratum of microsomes bounding the inner sphere. One of the granules remains behind as the centrosome of the new inner sphere ; which one of them is, apparently, determined entirely by position. The outer sphere has developed during this process.

The black granules in the inner sphere of Fig. 7*E* are plainly much less in bulk than those of Fig. 7*B*, *e.g.* There is no doubt that a large part of the centrosome granules has been changed into the red-staining substance of the sphere, which is identical in all noticeable respects with the substance from

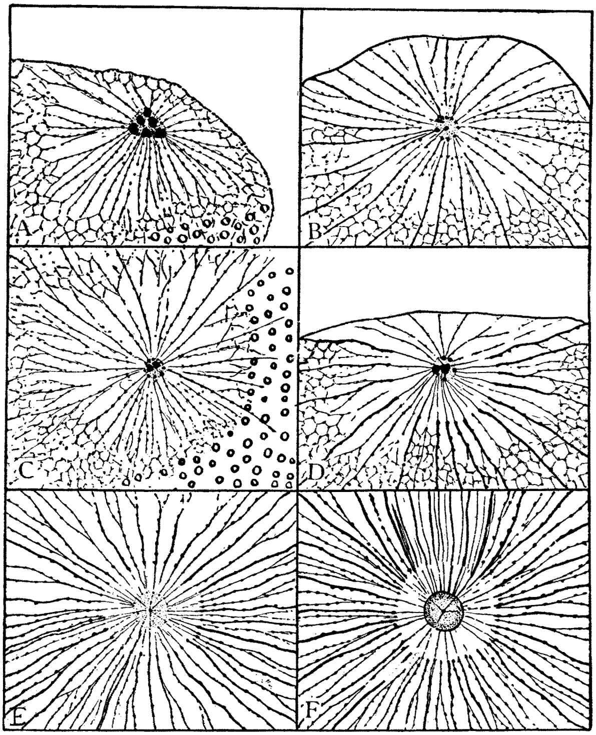

FIG. 7.—*A-E* are horizontal sections through the outer aster of the second maturation spindle, illustrating the phases of the centrosome from a stage slightly before that of Fig. 5 to the metaphase. *B*, *C*, and *D* are not necessarily successive stages, but simply different conditions met with between stages *A* and *E*. *F* is from a radial section of the inner aster in the beginning of the anaphase; this condition of the centrosome follows immediately 7*E* and precedes the stage of Fig. 6.

which the central spindle was formed. And in a later stage one sees the fibers of the central spindle dotted with large black microsomes.

From this description it would seem to follow that the centrosome of one cell-generation becomes the inner sphere of the next; and this is undoubtedly true at times. But I do not

believe that the inner sphere has *necessarily* any such definite morphological value as this would seem to imply. For it may disappear between the first and second maturation divisions, and is then reformed, as the first step in the prophase of the second maturation spindle, from the cytoplasm. The same method of formation may also be observed in other places (*e.g.*, formation of the first cleavage spindle).

Both Van Beneden's and Boveri's conceptions of the structure of the aster appear as phases in the history of the mitosis, though Boveri's "centrosome" is really the inner sphere, and his "Centralkorn" or "centriole" really the centrosome.

In conclusion, I shall sum up the evidence which the study of the egg of Unio has furnished against the theory of the permanency of the centrosome as a unique organ of the cell, combining an earlier paper[1] with this.

1. A sperm amphiaster is formed, but it disappears utterly at the time of the metaphase of the first maturation spindle.

2. Entirely independently of the sperm and egg asters, there arises in the egg of Unio at the time of the metaphase of the second maturation spindle an *accessory aster*, in the center of which is a minute centrosome. This centrosome divides and a small amphiaster is formed, which entirely disappears at the beginning of the telophase.

3. After the formation of the second polar globule the egg centrosome disappears.

4. The two cleavage centrosomes arise independently of any of their predecessors, and apparently separately.

5. Fission products of the centrosome become cytomicrosomes.

Thus the egg of Unio furnishes evidence : in the first place, that the centrosomes are not necessarily genetically continuous ; in the second place, that a centrosome may arise in the general cytoplasm (accessory aster) ; and, in the third place, that products of the centrosomes may become other formed elements of the cell. If this last centrosome phenomenon should

[1] Lillie, Frank R., "On the Origin of the Centers of the First Cleavage Spindle in Unio." See report of the meeting of the American Morphologists in Boston in *Science*, vol. v, p. 114. March, 1897.

be shown to be of general occurrence, it would place in a new light and on a new basis the theory that the cell is composed of self-perpetuating units of a lower rank. So far as these observations go, they tend to confirm Watasé's[1] theory, that centrosomes and cytomicrosomes are homologous structures.

Boveri, Brauer,[2] Van der Stricht,[3] Watasé,[4] Haecker,[5] McMurrich,[6] MacFarland,[7] and Klinckowström[8] have figured structures resembling in some respects the vesicular inner sphere here described. But all have designated them centrosomes. Haecker, and McMurrich have not figured the true centrosome at all.

McMurrich's notice, though brief and incomplete, is of especial interest, because he finds that the vesicular sphere is preceded by a group of deeply staining granules, from which it is probably derived. *How,* he does not know. He figures, moreover, the peripheral microsomes of the inner sphere as nodes in a network. From these nodes the radiating fibers arise. The contents of the inner sphere ("centrosome") are "perfectly homogeneous."

MacFarland's figures are more like mine than any others in the literature of the subject. Moreover, he has followed through exactly the same period with which I have dealt. But his conclusions are radically different, owing to his interpretation of the inner sphere as centrosome. With this interpretation goes his proof of Heidenhain's "Centralspindel und Centrosomen bilden der Genese nach ein Ganzes."

Some of Von Klinckowström's figures of the egg of Prosthe-

[1] Watasé, S., "Homology of the Centrosome," *Journ. of Morph.*, vol. viii, No. 2, 1893. See also *Science*, 1897.

[2] Brauer, A., "Zur Kenntniss der Spermatogenese von Ascaris Megalocephala," *Archiv f. mikr. Anat.* Bd. xliii. 1893.

[3] Van der Stricht, O., "De l'origine de la figure achromatique de l'ovule en mitose chez la Thysanozoon brocchi," in *Verh. Anat. Ges.* 1894.

[4] Watasé, S., "Homology of the Centrosome," *Journ. of Morph.*, vol. viii, No. 2, 1893, p. 434, figure of one of the maturation spindles of the egg of Unio.

[5] Haecker, *v. ante.*

[6] McMurrich, *v. ante.*

[7] MacFarland, *v. ante.*

[8] Von Klinckowström, A., "Beiträge zur Kenntniss der Eireifung und Befruchtung bei Prostheceraeus vittatus," *Archiv f. mikr. Anat. und Entw.* Bd. xlviii, Heft 4. 1897.

ceraeus show a similar vesicular inner sphere ("centrosome" K.). There are generally figured within the sphere a number of granules ; but in regard to this point one feels that celloidin sections 15μ thick and stained in borax carmine are inadequate evidence. However, in some eggs in an earlier stage he speaks of a minute "centriole" within the "centrosome."

To the criticism that the centrosome phases shown in Fig. 7*A–F* are pathological, *i.e.*, due to imperfect extraction of the haematoxylin or other action of the reagents, it may be replied : first, that they are found with different killing fluids ; second, that the changes are perfectly uniform in all cases, so that, knowing the stage of development of the spindle, one can be certain that a definite stage of the centrosome will be found ; third, that, inasmuch as the inner aster develops much more rapidly than the outer, the inner centrosome passes through these phases much more rapidly than the outer. Thus one often finds a spindle in which the inner centrosome has already reached the condition of Fig. 7*E*, while the outer is in the stage of 7*A* (*v.* Fig. 5). Now, 7*E* is a perfectly normal and typical centrosome ; hence, if one uses the pathological argument, one must be prepared to assert that one end of a spindle may be pathological and the other normal *in the same section.*

Those who oppose the centrosome theory are often met with the reply, "one positive observation is worth a great many negative ones" ; the implication being that one observation in favor of the permanency of the centrosome is worth a great many against it. I think that we are all convinced of the difficulty of proving a negative proposition. Nevertheless, it is a confession of weakness to dismiss all observations incompatible with a theory with the above caption. The observations against the permanency of the centrosome as a unique organ of the cell are now so many that they demand attention. One has a right to ask in detail how the theory is to be upheld against the foregoing observations and against others, such as Mead's,[1] that the two centers of the first maturation spindle are selected,

[1] Mead, A. D., "The Origin of the Egg Centrosomes," *Journ. of Morph.*, vol. xii, No. 2, 1897.

so to speak, from a large number of active ones ; as Morgan's,[1] that any considerable area in the sea-urchin egg may produce an aster, and I may add a centrosome, under the stimulus of abnormal salt content of the sea water ; or Mead's[2] observations in the same direction in Chaetopterus ; or Conklin's[2] observations on the egg of Crepidula, similar to mine ; or of the observations of Strasburger[3] and pupils that karyokinesis in vegetable tissues does not necessarily imply the presence of centrosomes at the poles of the spindle, as it does not in the maturation of Ascaris or of Molgula[3] (Crampton).

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January 6, 1898.

[1] Morgan, T. H., "The Production of Artificial Astrospheres," *Archiv f. Entwickelungsmechanik der Organismen.* Bd. iii, Heft 3. 1896. See also report in *Science* ('98) of the meeting of the American Morphologists in Ithaca in December, 1897.

[2] See report in *Science* ('98) of the meeting of the American Morphologists in Ithaca in December, 1897.

[3] *Jahrb. wiss. Bot.* Bd. xxx.